Environmentally Responsive and Reversible Regulation of Epidermal Barrier Function by γδ T Cells

Michael Girardi[1], Julia M. Lewis[1], Renata B. Filler[1], Adrian C. Hayday[2] and Robert E. Tigelaar[1]

The intraepithelial lymphocyte (IEL) network possibly composes the largest T-cell compartment in the body, but it is poorly understood. IELs show limited T-cell receptor (TCR) diversity and have been proposed to respond to generic stress signals rather than pathogen-specific antigens. Consistent with this, skin-resident TCR$\gamma\delta^{+}$ cells, known as dendritic epidermal T cells (DETC), downregulate cutaneous inflammation, promote wound healing, and protect against cutaneous neoplasia. These pleiotropic effects collectively suggest that DETC (and IEL more generally) may contribute to epithelial maintenance and barrier function. The present studies test this hypothesis. Using skin surface impedance analysis to measure hydration status and transepidermal water loss, we show that the epidermal barrier is defective in γδ T-cell deficient mice. However, this does not represent a constitutive role of γδ cells, but rather one that is dependent on environmental challenge, consistent with the primary role for lymphocytes being the response of the host to its environment. Likewise, the importance of the physiologic DETC-associated TCR is demonstrated by showing that V$\gamma5^{+}$ fetal thymocytes reconstitute the barrier function defect in TCR$\delta^{-/-}$ mice, while V$\gamma5^{-/-}$ mice also show environmentally responsive defects in cutaneous physiology.

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INTRODUCTION

T cells can be divided into two major subsets based on the expression of either of two heterodimeric T-cell receptors (TCR): αβ or γδ. While αβ T cells predominate in the blood and lymphatics, γδ T cells are preferentially enriched within various epithelial tissues (eg skin, genitourinary tract, and intestinal tract) situated at the junction of the host with its environment (Girardi and Hayday, 2005). These intraepithelial lymphocytes (IEL) are strongly implicated in providing and/or regulating the first line of defense against environmental insults including infectious agents (Roberts *et al.*, 1996), and chemical mutagens (Girardi *et al.*, 2001, 2003).

Since IEL express TCR of limited diversity (Asarnow *et al.*, 1989), they have long been proposed to function by recognizing and eliminating metabolically stressed epithelial cells (Janeway, 1988). However, the definitive identification of TCR ligands for γδ IEL has not been readily forthcoming (Steele *et al.*, 2003; Aydintug *et al.*, 2004), and the importance of the TCRs in particular aspects of IEL function has remained unclear.

A tractable experimental model for IEL function is provided by the dendritic epidermal T cells (DETC) of the skin, which form a network of morphologically distinct γδ T cells with almost uniform expression of an invariant Vγ5Vδ1 TCR (Asarnow *et al.*, 1989; Tigelaar *et al.*, 1990). Investigations by us and others have shown DETC to recognize and kill transformed keratinocytes (Havran *et al.*, 1991), to decrease tumor development in several models of cutaneous malignancy (Girardi *et al.*, 2001, 2003), to promote cutaneous wound healing (Jameson *et al.*, 2002), and to protect against potentially overexuberant αβ T-cell-mediated inflammatory responses (Girardi *et al.*, 2002). Collectively, these data suggest that DETC, and IELs more generally, exert pleiotropic effects in regulating local tissue integrity. Nonetheless, important questions remain, including whether DETC and other IELs achieve these effects by acting as constitutive regulators of epithelial integrity and resistance to perturbation, as has been suggested (Komano *et al.*, 1995), or whether they are primarily responsive to their environment, as are other T cells. This issue is addressed in this study by the analysis of epidermal barrier function in TCR$\delta^{-/-}$ mice housed under different conditions. Even subtle compromises in epidermal integrity can be reliably detected by measuring skin surface impedance (SSI) – where the instantaneous SSI serves as a measure of surface hydration, and the change in SSI over time (ie rise in slope) is a correlate of transepidermal water loss (TEWL) (Rougier, 1994; Segre *et al.*, 1999).

The association of an invariant γδ TCR (Vγ5Vδ1) with the DETC repertoire has been hypothesized to reflect a critical

[1]*Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, USA and* [2]*Peter Gorer Department of Immunobiology, GKT Guy's Hospital, London, UK*

Correspondence: Dr Michael Girardi, Department of Dermatology, Yale University School of Medicine, 333 Cedar Street, HRT 616, New Haven, Connecticut 06520-8059, USA. E-mail: girardi@yale.edu

Abbreviations: DETC, dendritic epidermal T cells; DPM, Derma Phase Meter; E17, embryonic day 17; IEL, intraepithelial lymphocytes; IVH, individually ventilated housing; SSI, skin surface impedance; TCR, T-cell receptor; TEWL, transepidermal water loss; TPA, D12-O-tetradecanoylphorbol-13-acetate

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interaction of DETC with a skin-specific ligand. Consistent with this, TCR$\delta^{-/-}$ mice harbor skin-associated TCR$\alpha\beta^{+}$ IELs (Jameson *et al.*, 2004), but clearly they do not substitute for TCR$\gamma\delta^{+}$ DETC in regulating inflammation. Moreover, Vγ5Vδ1^{+} T-cell progenitors will reconstitute the defects in regulating inflammation in TCR$\delta^{-/-}$ mice (Girardi *et al.*, 2002). Nonetheless, V$\gamma5^{-/-}$ mice, which harbor a large TCR$\gamma\delta^{+}$ DETC population, but that cannot express the physiologic DETC TCR, have not been assessed for their functional phenotype.

In sum, the data show that both TCR$\delta^{-/-}$ and V$\gamma5^{-/-}$ mice are defective in the physiologic regulation of cutaneous integrity, albeit that the former mice are affected more severely. Hence, the skin-associated TCR is a specific determinant of the functional competence of skin-resident IELs. Nonetheless, the defects in neither TCR$\delta^{-/-}$ nor V$\gamma5^{-/-}$ mice are constitutive, but are reversibly induced by environmental challenge. These data identify the murine cutaneous IEL subset as regulating epithelial integrity in response to the environment, and provide insight into the potential role of local T cells in relationship to epithelial diseases, such as atopic dermatitis.

RESULTS

TCR$\delta^{-/-}$ mice demonstrate defective epidermal barrier function

To determine whether γδ cells regulate epidermal barrier function *in vivo*, skin surface impedance (SSI) analysis (Rougier, 1994; Segre *et al.*, 1999) was performed on TCR$\delta^{-/-}$ mice, genetically deficient in all γδ T cells, and compared to normal controls (Figure 1). Instantaneous (t_0) SSI, a parameter of stratum corneum hydration status, was significantly higher in the ear skin of γδ-deficient mice (133.3±8.6 *vs* 110.3±1.9; $P=0.007$). In addition, the change in SSI over time ($t_{0-10\,s}$) was increased (>6-fold) in the ear skin of TCR$\delta^{-/-}$ mice (6.2±1.7 *vs* 1.0±0.1; $P=0.002$), indicating a major defect in the ability to protect against TEWL. To determine if a similar effect was present on a hair-bearing site, abdominal skin was razor shaved and assayed 24 hours later. Albeit to a lesser extent, the TCR$\delta^{-/-}$ mice again demonstrated significantly greater surface hydration (150.4±3.6 *vs* 129.2±1.6; $P=0.007$) and TEWL (3.6±0.5 *vs* 2.2±0.4; $P=0.03$).

Environmentally responsive epidermal defects in TCR$\delta^{-/-}$ mice

TCR$\delta^{-/-}$ and control mice were housed under different conditions in order to distinguish whether the effects of γδ cells on epithelial physiology were constitutive, as has been suggested (Komano *et al.*, 1995), or a reflection of the γδ response to the environment. Individually ventilated housing (IVH) provides a direct ventilation hook-up to each cage, maintaining relatively dry cage bedding with low ammonia levels, by comparison to conventional nonventilated housing (CNVH). Importantly, TCR$\delta^{-/-}$ and control mice do not demonstrate differences in baseline ear thickness or barrier function under IVH conditions (Figure 2a and b). However, relative to TCR$\delta^{-/-}$ mice in CNVH, those TCR$\delta^{-/-}$ mice maintained from birth in IVH (Group 1) showed markedly lower surface hydration (156.8±17.1 *vs* 309.8±34.6; $P<0.005$), TEWL (5.0±0.4 *vs* 12.8±1.0; $P<0.00001$), as well as a lower baseline ear thickness (Figure 2a–c). The housing conditions of the two TCR$\delta^{-/-}$ groups were then exchanged such that the mice originally maintained in the CNVH were moved to IVH, and *vice versa*. Strikingly, after only 7 days, the TCR$\delta^{-/-}$ mice moved from IVH to the CNVH demonstrated relatively higher surface hydration (157.4±13.8 *vs* 101.4±3.7; $P<0.005$) and TEWL (6.4±1.2 *vs* 1.4±0.4; $P<0.005$; Figure 2d), demonstrating that the role of γδ T cells in regulating barrier function is evident under environmentally stressful conditions.

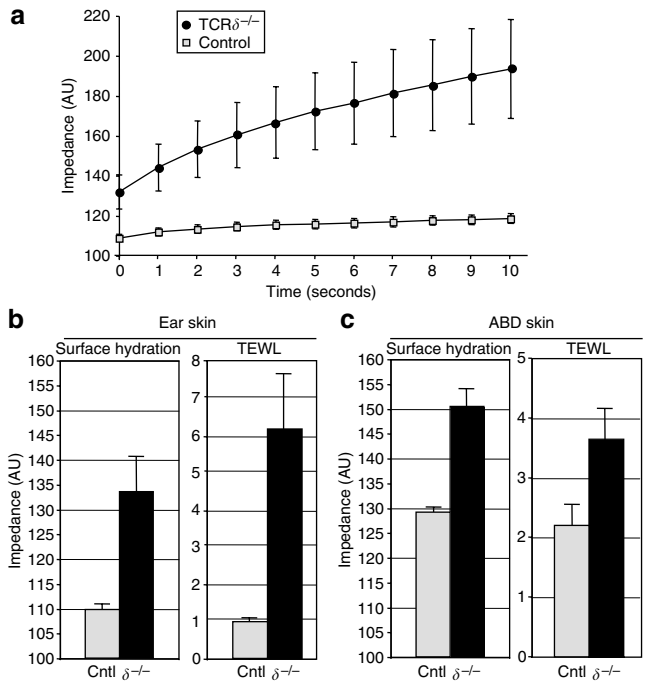

Figure 1. TCR$\delta^{-/-}$ mice demonstrate compromised epidermal barrier function. (**a**) The ear skin of TCR$\delta^{-/-}$ mice, deficient in all γδ T cells, demonstrated markedly higher skin surface impedance measurements over time. (**b**) From this analysis, the instantaneous impedance, indicative of surface hydration status, as well as the change in surface impedance over time, indicative of TEWL, were both significantly elevated in the ear skin of γδ-deficient mice. (**c**) Similarly, surface hydration and TEWL were significantly higher on abdominal skin, 24 hours after shaving the hair. AU, arbitrary units of impedance using the Nova DPM 9003; cntl, age- and strain-matched control mice.

Molecular parameters of γδ cell effects in murine ear skin

To gain a more detailed understanding of the effects of γδ cell deficiency on the skin, the ear skins of four TCR$\delta^{-/-}$ mice and four matched (12-week, non-obese diabetic (NOD)/Lt) cagemate control mice were analyzed using Operon Mouse OMM13K Arrays. In all, 31 (~0.1%) of the >13,000 genes were ⩾10-fold overexpressed in the TCR$\delta^{-/-}$ ears (Table 1). These genes are appreciably enriched in those encoding proinflammatory molecules (eg calgranulin B, IL-22, IL1β, chemokine C–X–C type ligand 2, C–X–C type ligand 5, acute-phase reactants (eg defensin β3, serum amyloid A2 and A3, immunoresponsive gene 1), and small proline-rich proteins

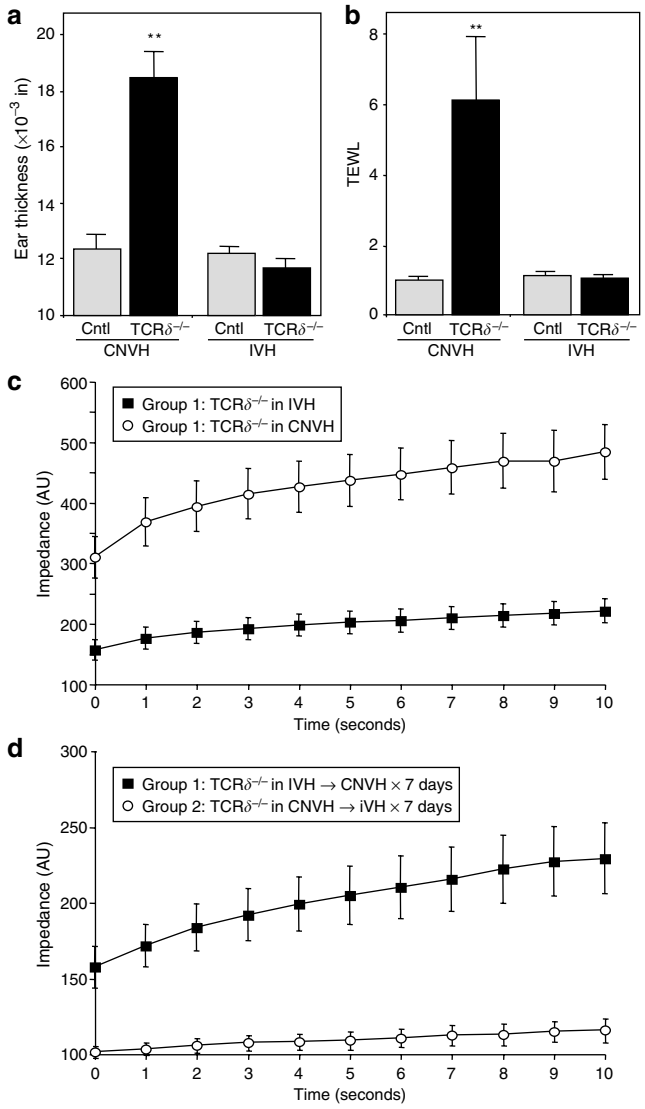

Figure 2. Epidermal barrier defect in TCR$\delta^{-/-}$ mice is dependent on environmental conditions. The ear skin of TCR$\delta^{-/-}$ mice demonstrated a significantly increased each (**a**) thickness and (**b**) TEWL when housed in conventional nonventilated cages, but not in individually ventilated cages, relative to controls. (**c**) A direct comparison of TCR$\delta^{-/-}$ mice under the two different housing conditions revealed markedly higher defects in barrier function in the CNVH mice. (**d**) Within 7 days of exchanging the environment of the two groups of mice, the mice originally housed under IVH conditions newly demonstrated a relatively higher defect in barrier function. $^{**}P<0.005$.

(Sprr1B, Sprr2H) – cornified envelope components known to be upregulated in compromised epithelial tissues (Mischke *et al.*, 1996). In addition, the 60-fold overexpression of urate oxidase may reflect the role of uric acid in provoking immune responses to dying cells (Shi *et al.*, 2003). Among the other overexpressed genes were three encoding transporters. In sum, the molecular analysis provides striking validation of the inflammation and altered barrier function that characterize the skin in TCR$\delta^{-/-}$ mice, and it identifies potential specific molecular markers of this state (see Discussion).

V$\gamma5^+$ DETC function to maintain the epidermal barrier

DETC compose the prototypic unconventional T-cell compartment, expressing essentially a monoclonal TCR (Asarnow *et al.*, 1989). To examine the role of this TCR in the regulation of epidermal integrity, TCR$\delta^{-/-}$ mice were selectively reconstituted with V$\gamma5^+$ DETC using a fetal thymocyte transfer protocol, in which it has been shown that intraperitoneal inoculation of newborn T-cell-deficient mice with unsorted (Havran and Allison, 1990; Payer *et al.*, 1991) or V$\gamma5^+$ sorted (Girardi *et al.*, 2002) fetal thymocytes from normal donors results in repopulation of V$\gamma5^+$ DETC. Three groups of mice were established, and compared at 10 weeks of age by surface impedence analysis for epidermal barrier function: TCR$\delta^{-/-}$ mice reconstituted with unsorted thymocytes, or with V$\gamma5^+$ sorted thymocytes, showed significantly lower skin surface hydration and TEWL measurements than the control TCR$\delta^{-/-}$ mice (Figure 3). Thus, prototypic V$\gamma5^+$ DETC are sufficient to maintain epidermal barrier function.

Assessment of $\gamma\delta^+$ epidermal T cells in the absence of the prototypic V$\gamma5^+$ TCR

To determine whether prototypic V$\gamma5^+$ DETC are necessary to maintain epidermal barrier function, V$\gamma5^{-/-}$ mice were examined. These mice harbor a replacement population of V$\gamma5^-$ $\gamma\delta^+$ epidermal T cells with dendritic morphology, which variably include a fraction of cells reactive to the monoclonal antibody 17D1, which recognize the idiotypic portion of the prototypic V$\gamma5^+$ DETC TCR. This has led to the hypothesis that V$\gamma5^{-/-}$ mice reflect a pressure, albeit not absolute, for the development of DETC whose TCR expression is as close as possible to that of normal DETC (Mallick-Wood *et al.*, 1998; Hayday, 2000).

A small and significant increase in baseline ear thickness was observed in V$\gamma5^{-/-}$ mice relative to normal controls, although this was less than in the TCR$\delta^{-/-}$ mice (Figure 4a). Moreover, the skin dysregulation in V$\gamma5^{-/-}$ mice was amplified by skin sensitization (DNFB) and irritation (D12-*O*-tetradecanoylphorbol-13-acetate (TPA)), respectively (Figure 4d and e). Although there was no constitutive defect in TEWL in the V$\gamma5^{-/-}$ mice, there was a difference in surface impedence (Figure 4b and c), and, again, this difference was amplified by cutaneous irritation (Figure 4f).

DISCUSSION

Studies of various IELs subsets have demonstrated that these cells possess several overlapping features of both natural killer cells and T regulatory cells, as well as the capacity to produce growth factors with the potential to contribute to maintaining epithelial integrity (reviewed in Kabelitz and Wesch, 2003). The DETC in murine skin represent the prototypic IEL, where virtually all of the T cells express the identical V$\gamma5$V$\delta1$ TCR. The fact that TCR$\delta^{-/-}$ mice, in which a replacement population of TCR$\alpha\beta^+$ IEL are found throughout the epidermis, demonstrate defective wound healing (Jameson *et al.*, 2002), dysregulated cutaneous inflammation (Girardi *et al.*, 2002), and increased tumorigenesis (Girardi *et al.*, 2001) suggests that the prototypic DETC TCR recognizes a yet unidentified, stress-induced, cutaneous ligand.

Table 1. Genes showing 10-fold or greater expression in ears of γδ-deficient (TCRδ$^{-/-}$) mice

	Gene (gene symbol)	Rel. expr.	Putative role in TCRδ$^{-/-}$ mice
1.	Calgranulin B (s100a9)	69.7	IL-8 mediated inflammation
2.	Urate oxidase (Uox)	59.3	Purine metabolism
3.	Defensin β3 (Dfb3)	31.2	Anti-bacterial peptide released by KCs
4.	RNA BP (Rbm8)	27.7	Regulation of gene expression
5.	IL-22	29.5	IL-10-inducible T_H1 cytokine, induces expression of Defensin β3 (see 3 above)
6.	IL-1β (Il1b)	25.2	Primary cytokine released during epidermal stress/injury
7.	Serum amyloid A3	23.7	Acute-phase reactor
8.	IL-1β (Il1b)	23.6	Array repeat control; see 6 above
9.	Pendrin (Slc26A4)	23.0	Solute transporter
10.	Small proline-rich protein 2H	21.7	Unknown
11.	RIKEN cDNA 2310007F0	20.8	Unknown
12.	RIKEN cDNA 2610014H22	20.9	Unknown
13.	Serum amyloid A3	20.3	Array repeat control; see 7 above
14.	RIKEN cDNA 4930505H01	17.1	Unknown
15.	Immunoresponsive gene 1 (Irg1)	17.0	LPS-inducible gene of unknown function
16.	CXCL-2 (Cxcl2)	15.6	PMN chemotaxis and transmigration
17.	CXCL-5 (Cxcl5)	14.9	PMN chemotaxis and transmigration
18.	Hemoglobin-β	14.5	May reflect vascular dilatation
19.	Fetuin-β	13.7	Unknown; extracellular space protein
20.	Insulin-like growth factor-BP2	12.7	Putative cellular growth factor
21.	Hyaluronan synthase 3 (Has3)	13.1	Synthesis of extracellular matrix
22.	Eosinophil-associated ribonuclease 5	11.5	Unknown
23.	Serum amyloid A2 (Saa2)	11.3	Acute-phase reactor
24.	RIKEN cDNA 9030605I04	11.0	Unknown
25.	Regenerating islet-derived 3γ (Reg3g)	10.6	Acute-phase reactor
26.	IL-18 receptor accessory protein	10.8	Putative regulator of IL-18
27.	Triggering receptor expressed on myeloid-1 (Trem1)	10.4	Activation of PMNs
28.	Solute carrier family 7, member 11	11.1	Cation transporter
29.	Potassium voltage-gated channel, member 1 (Kcnb1)	11.1	Epithelial electrolyte transport
30.	Small proline-rich protein 1B (Sprr1b)	10.0	Putative squamous differentiation factor
31.	Keratin 16 (Krt1-16)	10.0	Keratin of proliferative epidermis

BP, binding protein; CXCL, chemokine (C–X–C type) ligand; IL, interleukin; KC, keratinocyte; LPS, lipopolysaccharide; PMN, polymorphonucleocytes; T_H1, T-helper type-1.

While no human correlate of DETC has been identified, γδ T cells of limited diversity have been described in human dermis (Holtmeier *et al.*, 2001). Hence, murine skin offers the opportunity to better understand the role of the local immune system, and specifically IELs, in the protection of epithelial integrity

An intriguing report by Komano *et al.* (1995) led the authors to conclude that intestinal IEL maintenance of the epithelium was a physiologic role, where IEL presumably continually produced local cytokines to facilitate epithelial turnover. However, the view of a lymphocyte population as homeostatic regulators of the epithelial barrier is unorthodox in comparison to the paradigm of conventional lymphocytes that become activated and exert their effector functions in direct response to a (e.g. antigenic) challenge. Conversely, in the midst of local immune responses, epithelial barrier function must be preserved to whatever extent is possible, and it therefore remains plausible that the effects of IELs on their local tissues reflect their activities in response to environmental challenges to the host. This perspective would more closely align IELs with activities of αβ T cells despite their fundamental differences in antigen recognition characteristics. These issues have been addressed here, and it is now clear that DETC function in the skin to maintain epidermal

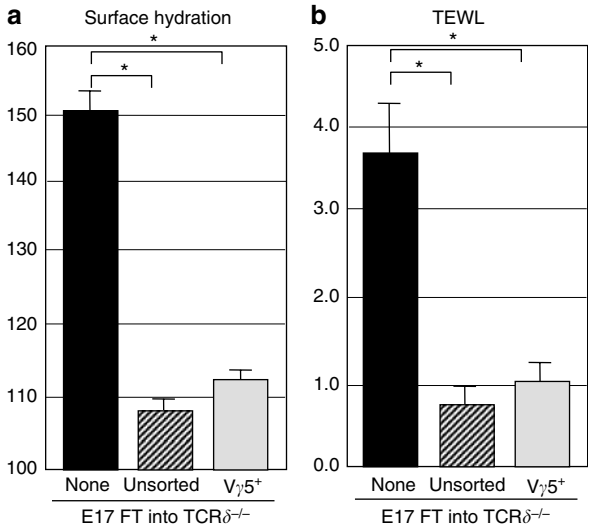

Figure 3. Vγ5$^+$ DETC mediate protection of the epidermal barrier. Both (**a**) surface hydration and (**b**) TEWL were significantly higher in TCRδ$^{-/-}$ mice relative to littermate groups of TCRδ$^{-/-}$ that had been neonatally reconstituted with Vγ5+ DETC by receiving either E17 unsorted or FACS-sorted Vγ5+ fetal thymocytes from normal donors (*P<0.001).

integrity, and that this function is intimately dependent on the DETC TCR and response to environmental perturbations.

These studies took advantage of the fact that impedence measurements at the cutaneous surface can be utilized to reliably detect abnormalities in epidermal barrier function, including TEWL (Rougier, 1994). The Nova Derma Phase Meter (DPM) 9003, sometimes referred to as a ''Novameter,'' is specifically engineered to assess epidermal barrier function via impedence measurements. The ear skin allowed for direct application of the sensor probe, without the need for removal of hair. Ear skin, without the protection provided by hair, might be expected to be exposed to more potential environmental irritants. Indeed, while significant, the difference in barrier function of abdominal skin in TCRδ$^{-/-}$ and control mice was smaller than for ear skin.

Expression analysis of the ear skin of TCRδ$^{-/-}$ mice, relative to normal controls, provides insight into the epidermal barrier defect in the absence of γδ T cells. Having previously reported that γδ-deficient mice develop a spontaneous dermatitis, we were not surprised to find that the majority of highly expressed genes in the skin of such mice are mediators of inflammation and/or so-called acute-phase reactancts. For example, in the skin of TCRδ$^{-/-}$ mice, the relatively most overexpressed gene, *calgranulin B*, has recently been shown to play a key role in IL-8-mediated airway hyper-responsiveness by lung epithelium (Ahmad *et al.*, 2003). Nonetheless, further studies (beyond the scope of the currently reported experiments) are necessary to understand the relationship of this and other variably expressed genes to the observed defect in epidermal barrier function.

DETC, the IELs of the skin, preferentially express an invariant Vγ5+ TCR, largely due to genetic control over TCR rearrangement in the fetal thymus. This results in a wave of

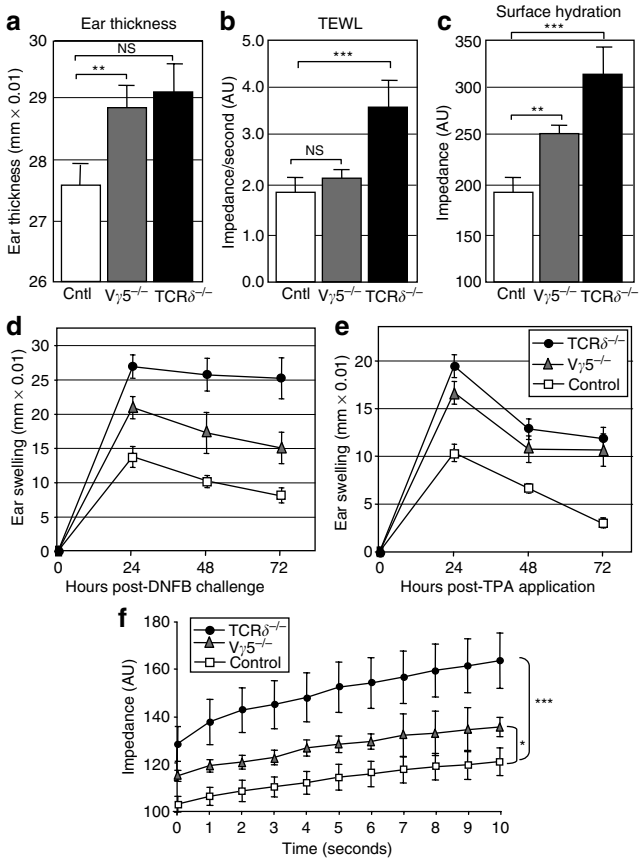

Figure 4. Protection of the epidermal barrier by γδ T cells is dependent in part on the prototypic DETC Vγ5 TCR. (**a**) Baseline ear thickness (a parameter of inflammation) in Vγ5$^{-/-}$ mice was significantly higher than controls, and approximated the level of TCRδ$^{-/-}$ mice. Vγ5$^{-/-}$ mice were assessed for epidermal barrier function by surface impedance, and compared to syngeneic controls as wells as TCRδ$^{-/-}$ mice. While (**b**) surface hydration was significantly elevated in the Vγ5$^{-/-}$ mice relative to normal controls, (**c**) TEWL was essentially normal. In addition, when assayed by (**d**) elicitation to allergen DNFB, or (**e**) application of irritant TPA, the Vγ5$^{-/-}$ mice demonstrated significantly elevated ear swelling responses relative to controls. (**f**) The impedance analysis for Vγ5$^{-/-}$, TCRδ$^{-/-}$, and control mice is shown 24 hours after TPA application, with the Vγ5$^{-/-}$ mice demonstrating readings intermediated between the TCRδ$^{-/-}$ and control mice. Taken together, the data indicate that γδ T cell-mediated protection of the epidermal barrier as measured in several assays is dependent in part on TCR Vγ5, but that other γδ T cells may be operative in the absence of the prototypic DETC TCR (*P<0.05, **P<0.01, ***P<0.005; relative to controls).

Vγ5+ DETC precursors in the day 14 to 17 fetal thymus, which allows for the selective repopulation of TCRδ$^{-/-}$ mice with prototypic Vγ5+ DETC using a fetal thymus transfer protocol. Thus, we were able to determine that indeed Vγ5+ prototypic DETC were capable of mediating protection of the epidermal barrier. While Vγ5-deficient mice did demonstrate subtle differences in barrier function, especially after exposure to the strong irritant TPA, such mice have a replacement population of γδ T cells with potential barrier protective activity (Mallick-Wood *et al.*, 1998). Nonetheless, the augmented cutaneous inflammation determined by ear-swelling response (eg irritant dermatitis and allergic dermatitis) of Vγ5$^{-/-}$ relative to normal controls indicates that the

prototypic DETC Vγ5+ TCR is important to the functionality of this locally resident T-cell population.

The defect in epidermal barrier function in γδ-deficient mice provides yet another parallel of γδ-deficient mice to human disorder atopic dermatitis. In addition to abnormal barrier function, the skin of TCR$\delta^{-/-}$ mice and the skin of atopic dermatitis show a similar histopathology, and dependence on environmental influences, resulting in a localization of lesions to sites more apt to be exposed to chemical irritants or allergens (Nassif *et al.*, 1994). In the absence of αβ T cells (as in TCR$\beta^{-/-}\delta^{-/-}$ mice), the defect in barrier function attributable to the absence of γδ T cells is not observed, suggesting that γδ T cells protect the epidermis by local control of αβ T cells, which may otherwise contribute to inflammation and barrier disruption. This is consistent with the hypothesis that atopic dermatitis is a disorder of insufficient local immunoregulation (Hayday and Tigelaar, 2003). Since scratching contributes substantially to the ear-swelling response of susceptible mice, future experiments in which the mice may be prevented from scratching may give additional insight into whether prototypic DETC protect the epidermal barrier by acting directly on keratinocytes or primarily through inhibition of inflammatory-driven pruritus.

In summary, the presented studies of DETC function in mice indicate that the local immune system is essential for protection of epidermal integrity, responding to environmentally induced perturbations. The parallels of the findings in the skin of mice deficient of $\gamma\delta^+$ IEL to inflammatory cutaneous disease in humans further heighten the potential importance of efforts to identify IEL self-ligands and increase our understanding of any potential homologous local immune effects in the human state.

MATERIAL AND METHODS

Animals and housing

TCR$\delta^{-/-}$ (Itohara *et al.*, 1993) were purchased from Jackson Laboratories (Bar Harbor, ME). V$\gamma5^{-/-}$ were produced as described previously (Mallick-Wood *et al.*, 1998). These mice have been characterized as phenotypically normal, with normal Langerhans cells and a replacement population of $\gamma\delta^+$ V$\gamma5^{(-)}$ epidermal T cells with dendritic morphology. Each mutant was backcrossed 10+ generations onto the FVB/N background, and maintained as local colonies under pathogen-free conditions in micro-isolator cages with autoclaved food and bedding. In indicated experiments, selected groups of mice were kept in CNVH, while others were maintained in IVH. All of the *in vivo* studies were approved by the Yale Animal Care and Use Committee.

Skin surface impedance

SSI was measured using a Nova DPM 9003 (Nova Technology Corp., Portsmouth, NH) with a 5-mm-diameter DPM 9123X custom probe. The DPM device produces values of skin surface impedance expressed as arbitrary units (range 90–999), which in the skin is a correlate of capacitance (http://www.novatechcorp.com/measure.html). Skin surface hydration was determined as the instantaneous (t_0) impedance reading at probe application to the skin. TEWL was determined in continuous mode as the change in SSI over the 10-seconds interval after probe application (Rougier, 1994; Segre *et al.*, 1999).

Contact dermatitis response

All chemicals were obtained from Sigma Chemicals, St. Louis, MO. Ear thickness measurements were performed using a Mitutoyo No. 7301 engineer's micrometer (Mitutoyo America Corp., Aurora, IL). To induce allergic contact dermatitis, mice were sensitized on day 0 by epicutaneous application to razor-shaved abdominal skin of 25 μl of 0.5% DNFB in a mixture of acetone:olive oil (4:1). On day 5, after measuring baseline ear thickness with an engineer's micrometer, mice were challenged by applying 10 μl of 0.2% DNFB in acetone:olive oil to each side of each ear. For irritant contact dermatitis assays, after measuring baseline ear thickness, 20 nmol TPA (in 10μl acetone) was applied to each side of each ear of naïve adult mice. Ears were re-measured 24, 48, and 72 hours after challenge; data are expressed as the ear-swelling response above baseline (ie ear thickness 24 hours after challenge *minus* ear thickness immediately before challenge) ± 1 standard error of the mean.

Expression analysis

Mouse ears were snap frozen in liquid nitrogen, and pulverized into powder in liquid nitrogen using mortar and pestle. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), following the manufacturer's protocol. RNA was further purified by phenol:chloroform:isoamyl alcohol extraction and precipitated using 5 M ammonium acetate and 100% ethanol. RNA pellet was resuspended in diethyl pyrocarbonate water and mRNA was isolated using an Oligotex mRNA kit (Qiagen, Valencia, CA). The concentration and purity of mRNA were verified by a spectrophotometer and by 1.1% formaldehyde gel. Yield of mRNA was 1.5% of total RNA. Obtained mRNA was processed and analyzed by the Yale University WM Keck Foundation Biotechnology Resource Laboratory using Operon Mouse OMM13K arrays.

Reconstitution of TCR$\delta^{-/-}$ mice with V$\gamma5^+$ DETC

Previously, we have shown that embryonic day 17 (E17) fetal thymus contains V$\gamma5^+$ DETC precursors, which upon adoptive transfer reconstitute V$\gamma5^+$ DETC in the skin of DETC-deficient TCR$\delta^{-/-}$ recipients (Girardi *et al.*, 2002). Briefly, a single-cell suspension of fetal thymocytes was prepared in Hank's balanced salt solution from thymi obtained from E17 FVB/N fetuses obtained after timed matings. Red blood cells and dead cells were removed by Lympholyte M (Accurate, Westbury, NY) density gradient centrifugation. Thymocytes were then blocked with normal hamster IgG and anti-Fc receptor (FcR) (2.4G2; Pharmingen, San Diego, CA), stained with anti-Vγ5 (FITC-F536; Pharmingen) or isotype-matched control, and sorted for V$\gamma5^+$ expression on a FACSVantage (Becton Dickinson, Mountain View, CA). One group of newborn TCR$\delta^{-/-}$ mice received intraperitoneal inoculi of two fetal thymus equivalents (2×10^6) of unsorted E17 fetal thymocytes, one group

received two fetal thymus equivalents (8×10^4) of flow-cytometry-sorted (>98% pure) $V\gamma5^+$ E17 fetal thymocytes, and a third group was left as unreconstituted $TCR\delta^{-/-}$ controls.

Statistics

Statistical significance was evaluated by the two-tailed, unpaired Student's *t*-test, or nonparametric analysis if standard deviations were significantly different between the two compared groups.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

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